

Amendments to the Specification:

Please replace the paragraph beginning at page 10, line 26, with the following rewritten paragraph:

C1
-- The recombinant cells of the invention contain at least one heterologous gene that encodes a glycosyltransferase or other enzyme that is involved in oligosaccharide synthesis. Many glycosyltransferases are known, as are their polynucleotide sequences. *See, e.g.*, "The WWW Guide To Cloned Glycosyltransferases, " (~~http://www.vei.co.uk/TGN/gt_guide.htm~~) (www.vei.co.uk/TGN/gt_guide.htm). Glycosyltransferase amino acid sequences and nucleotide sequences encoding glycosyltransferases from which the amino acid sequences can be deduced are also found in various publicly available databases, including GenBank, Swiss-Prot, EMBL, and others. --

Please replace the paragraph beginning at page 31, line 16, with the following rewritten paragraph:

C2
-- The elucidation of the nature of the oligosaccharide receptors is an ongoing endeavour, and more receptors for toxins and adhesins are being defined as time goes on. Selecting appropriate carbohydrate structures can be achieved by searching through a database of known carbohydrate structures, such as Carbbank, which is available over the internet; CDrom versions are also available from NBRF, National Biomedical Research Foundation, 3900 Reservoir Road, NW, Washington DC 20007 USA. Selecting appropriate nucleic acid sequences for expression of the desired glycosyl transferases can be achieved by searching through a database of genes encoding glycosyl transferases available over the internet, such as CAZy. This database is administered by AFMB-CNRS a contact being at 31 Chemin Joseph Aiguier F-13402 Marseille Cedex 20 (France). An alternative is to search for structures in the Chemical Abstracts. The search concerned will identify the sugar specificity of the transferase, the sugar to which it binds, the nature of the bond, and the overall nature of the acceptor molecule. Thus where the acceptor molecule is a LPS then a transferase specific for LPS will be preferred. The gene encoding the transferase of interest can be either made synthetically or alternatively the gene may be isolated from an appropriate organism either by direct cloning methods or by PCR amplification methods

12 and incorporated into an expression vector. A database suitable for searching of enzymes that may be used to provide for nucleotide precursors such as epimerases, dehydrogenases, transmutases and the like can be found at the following internet address:

<http://wit.mcs.anl.gov/WIT2/> wit.mcs.anl.gov/WIT2/. --

Please replace the paragraph beginning at page 56, line 1, with the following rewritten paragraph:

13 -- LPS was then purified from the above strain as well as from *E. coli* CWG308 and CWG308:pJCP-Gb3 and analysed by SDS-polyacrylamide gel electrophoresis with silver-staining as previously described (64). Whilst there was a clear difference in mobility of the LPS from CWG308 and CWG308:pJCP-Gb3, expression of the additional transferase gene in CWG308:pJCP-*lgtCDE* did not further retard LPS mobility (Figure 5). This could be explained either by failure to produce functional LgtD or by absence of the essential precursor UDP-GalNAc. This would require a functional UDP-GalNAc-4-epimerase, an enzyme not necessarily present in all *E. coli* strains. In a previous study (49) we described the genetic locus for biosynthesis of *E. coli* O113 O-antigen, the repeat unit structure of which includes GalNAc. This locus contains two genes (designated *gne* and *wbnF*) encoding proteins with similarity to nucleotide sugar epimerases and we postulated that one or other of these may be a functional UDP-GalNAc-4-epimerase. We therefore amplified the *gne* and *wbnF* genes from *E. coli* O113 chromosomal DNA using primers 5'-TTTATTAAGCTTCCAATTAAGG AGGTAAGTTC-3' (SEQ ID NO:16) and 5'-AATTACAAGCTTATAATTTTAATTACCA TACCC-3' (SEQ ID NO:17) for *gne* and primers 5'-ATATTCAAGCTTGAGTGAGGAT TATAAATGAAATT-3' (SEQ ID NO:17) and 5'-TTTCTTAAGCTTTTGTAATAATCAAA CTTTATAGAAG-3' (SEQ ID NO:18) for *wbnF* (each primer incorporates a *Hind*III site). Each PCR product was purified, digested with *Hind*III and ligated with *Hind*III-digested pJCP-*lgtCDE* and then transformed into *E. coli* JM109. Correct insertion and orientation of each construct (designated pJCP-*lgtCDE/gne* and pJCP-*lgtCDE/wbnF*) was confirmed by sequence analysis, and then each plasmid was transformed into CWG308. Comparison of the electrophoretic mobility of LPS purified from these recombinant strains (Figure 5) indicated that expression of the *gne* gene resulted in an increase in molecular size of the LPS. This gene was originally designated *galE*

C3
(15) because it encoded a product with a high degree of similarity to putative GalE proteins (UDP-Glc-4-epimerases) from a large number of bacteria, the most closely related being that from *Yersinia enterocolitica* O:8 (57% identity, 73% similarity) (23). However, the *Yersinia galE* gene is now designated *gne* on the Bacterial Polysaccharide Gene Database (available at ~~www.microbio.usyd.edu.au/BPGD/default.htm~~ microbio.usyd.edu.au/BPGD/default.htm) and the function of its product is listed as a UDP-GalNAc-4-epimerase. Given the high degree of similarity between the *Yersinia* and *E. coli* O113 proteins, and the fact that LgtD is a proven GalNAc transferase (33), we conclude that *galE* from the *E. coli* O113 *rfb* locus also encodes a functional UDP-GalNAc-4-epimerase, and accordingly it has been renamed *gne*. --

Please replace the paragraph beginning at page 62, line 25, with the following rewritten paragraph:

C4
-- GM1 is mimicked by the LPS outer core of several *Campylobacter jejuni* strains including the strain deposited as NCTC 11168. These are known to be capable of binding purified CT *in vitro* (59). The genome sequence of NCTC 11168 is available at http://www.sanger.ac.uk/Projects/C_jejuni ~~sanger.ac.uk/Projects/C_jejuni~~. Moreover, Linton *et al.* (60) have identified the LOS-encoding region within this sequence and have functionally characterized one of the genes (*wlaN*) involved in synthesis of the GM1 mimic LOS structure. As with some of the *Neisseria lgt* genes described previously, *wlaN* has a poly-G tract which will have to be mutagenized to stabilize expression, as described above for *lgtA*, *lgtC*, and *lgtD*. Sequence data for the *C. jejuni* LOS region will be used to design primers for PCR amplification of the appropriate genes for assembly of the GM1 mimic on the outer core LPS of CWG308. Additional CWG308 derivatives expressing the alternative LT-binding oligosaccharide epitopes listed in US Patent 6,069,137 can also be constructed. LPS can be extracted from each construct and analysed by SDS-PAGE as before. CWG308 derivatives can then be tested for binding and neutralization of purified CT and LT using a commercial ELISA assay or by direct blotting using commercially available peroxidase-conjugated CT. Protective capacity of any toxin-binding constructs can also be tested in an infant mouse cholera model. --
